502-Pos Board B282
How Half-Coated Janus Particles Enter Cells
Yuan Gao, Yan Yu.
Chemistry, Indiana University Bloomington, Bloomington, IN, USA.
Janus particles, the two-faced particles with different surface makeups on opposing sides, possess interesting properties such as functional asymmetry and directional anisotropy. As a result, Janus particles offer many promising biological applications not available with homogeneous particles. An in-depth understanding of how Janus principle affects cell-particle interactions, especially cellular uptake of particles, remains elusive at present yet is fundamental to engineer Janus particles of desired biological functions. In this study, by combining live-cell fluorescence imaging and quantitative analysis on a single-cell level, we will demonstrate how functional asymmetry dictates the cell membrane dynamics during Janus particle internalization. In addition, we will also discuss how to use Janus geometry to modulate cellular uptake of Janus particles.

503-Pos Board B283
Interactions of Liposomal Opa Proteins with Human Cell Surface Ceacam Receptors
Jason Kuhn.
Department of Chemistry, University of Virginia, Charlottesville, VA, USA.
Opacity-associated (Opa) proteins are β-barrel membrane proteins found on the surfaces of pathogenic *Neisseria gonorrhoeae* and *N. meningitidis*. These membrane proteins interact with specific human carcinoembryonic antigen-like cell adhesion molecule (CEACAM) receptors in order to induce bacterial phagocytosis into human host cells. Importantly, bacterial phagocytosis can even be induced in typically non-phagocytic cells, such as HeLa cells transfected to express CEACAM receptors. Opa variants interact with one or more members of the CEACAM family: CEACAM1, CEACAM3, CEACAM5, and CEACAM6, which are expressed on different tissue types and coordinate different signaling events during the phagocytic process. Using fluorescence microscopy, we aim to determine whether Opa proteins folded in liposomes can bind with CEACAM receptors on the surface of transfected HeLa cells as well as determine the fate of liposomes upon CEACAM-dependent cell surface interactions. The use of liposomes as membrane mimics enables us to study cellular Opa-CEACAM binding by bridging traditional biochemical and biophysical methods performed in liposomes or detergent micelles. The results indicate that Opa-proteoliposomes bind to CEACAM1 receptors on the surface of transfected HeLa cells. In addition, preliminary data suggest that Opa-proteoliposomes are engulfed into CEACAM1-transfected HeLa cells. These experiments support the hypothesis that liposomes can stabilize folded Opa proteins and retain cellular CEACAM binding.

504-Pos Board B284
Antibody Induced PLAP Endocytosis is Dependent on the Structure and Amount of Sterols in Cellular Plasma Membrane
Ji Hyun Kim, Deborah Brown, Erwin London.
Stony Brook University, Stony Brook, NY, USA.
Cell membranes are believed to contain lipid rafts which are ordered membrane domains rich in sphingolipids and sterols. Many studies investigating the functional role of lipid rafts are performed by removal of cholesterol from plasma membranes. However, it is uncertain whether the functional changes observed are due to altered raft levels or a more specific cholesterol-dependent effect. To distinguish these cases, we carried out sterol substitution experiments using methyl-β-cyclodextrin with a variety of raft formation-stabilizing and raft formation-destabilizing sterols. This approach was then applied to antibody-induced placental alkaline phosphatase (PLAP)-endocytosis. This process is cholesterol-dependent, but the mechanism of the cholesterol dependence is not known. Sterol depletion and substitution was carried out in MDA-MB-231 cells stably transfected with PLAP. After substitution, cholesterol-depletion and sterol-substitution were confirmed by HP-TLC. To define the effect of sterols on membrane structure TMADPH was added to the cells and fluorescence anisotropy measured. TMADPH anisotropy reflects plasma membrane lipid packing, and high values should be correlated with higher raft levels. Compared to untreated cells, cholesterol-depletion decreased anisotropy as expected. Subsequent replacement of cholesterol or dihydrocholesterol increased anisotropy to a similar or slightly higher level than that prior to depletion, consistent with restoration of raft levels, while anisotropy was somewhat lower with epicholesterol and much lower with lanosterol. Next, antibody-induced PLAP endocytosis was visualized by immunofluorescence. Only substitution with cholesterol restored PLAP internalization levels to an extent similar to untreated control. Decreasing cholesterol or substitution of cholesterol with other sterols inhibited internalization. This suggests that specific details of sterol structure are likely to be more important than tightness of lipid packing or raft levels in plasma membrane for antibody-induced PLAP endocytosis.

505-Pos Board B285
Cell Spreading Size Regulates Size of Clathrin-Coated Pits through Tension
Allen Liu, Xinyu Tan, Johanna Heureaux.
Mechanical Engineering, University of Michigan, Ann Arbor, MI, USA.
Intracellular organization depends on close communication between the extracellular environment and a network of cytoskeleton filaments. The interactions between cytoskeletal filaments and the plasma membrane lead to changes in tension that in turns help regulate biological processes. Endocytosis is known to be stimulated by low membrane tension and the removal of membrane increases membrane tension. While it is appreciated that the opposing effects of exocytosis and endocytosis have on keeping plasma membrane tension to a set point, it is not clear how tension affects the dynamics of clathrin-coated pits (CCPs), the individual functional units of clathrin-mediated endocytosis. Furthermore, although it was recently shown that actin dynamics counteracts membrane tension during CCP formation, it is not clear what roles membrane tension and cortical tension play in regulating different aspects of CCP dynamics. We designed micro-patterned surfaces of different sizes to control the cell spreading sizes which regulate tension. Total internal reflection fluorescence microscopy of living cells and high content image analysis were used to quantify the dynamics of CCPs. We found that increasing cell spreading increased CCP initiation and the proportions of short-lived, and likely abortive, CCPs. However, perturbations that aimed to alter membrane and cortical tension suggest a more complex regulation in CCP initiation and the checkpoint to CCP maturation beyond simply physical input. By analyzing fluorescence intensities of CCPs under different conditions, we discovered that cortical tension regulates the size of individual CCPs. Together, our study reveals new mechanistic insights into how plasma membrane tension regulates the dynamics of CCPs.

506-Pos Board B286
Synaptic Vesicle Turnover in Human Brain Synaptosomes
Arup R. Nath[1], Ileea Larente[1], Taufik Valiante[2], **Elise F. Stanley**[1].
[1]C&MB, TWRI, UHN, Toronto, ON, Canada, [2]Neurosurgery, TWH, UHN, Toronto, ON, Canada.
Current understanding of synaptic transmission in the human brain is based primarily by analogy with data obtained using animal model preparations with large presynaptic terminals from squid, chick, rat or mouse. While these models are excellent for the analysis of general principles of synaptic transmission they are unlikely to serve for the understanding of human-specific modifications or defects. To address this issue we have developed a human preparation based on fresh excised human brain tissue that permits the examination of synaptic vesicle recycling in identified single presynaptic terminals (synaptosomes, SSMs).
A plug of middle temporal gyrus cortex, removed for hippocampal epilepsy focus surgery (Valiante), was homogenized. SSMs were isolated and purified by micro-discontinuous sucrose gradient centrifugation. The isolation of SSMs was confirmed by immunocytochemistry and biochemistry (Western blot) using a wide range of presynaptic markers. Individual SSM types could be identified by immunostaining for postsynaptic receptors since a small piece of the postsynaptic membrane typically remains attached to the presynaptic nerve terminal (See: Nath et al., Fronteirs in Cellular Neuroscience 2014). The SSMs were functional as SV turnover was demonstrated in individual terminals by Ca^{2+}-triggered styryl-dye uptake and destaining. We also demonstrated that the SSMs could be frozen for later physiological study and loaded with intracellular test compounds using our Cryoloading method (ibid). This new method will provide a means to assess SV turnover and transmitter release in identified presynaptic nerve terminals in human brain and examine human-specific disorders such as Parkinson's and Alzheimer's diseases.

507-Pos Board B287
Controlling Stimulus-Secretion Coupling in Adrenal Chromaffin Cells: A Novel Role for the Serotonin Transporter?
Rebecca L. Brindley[1], Randy D. Blakely[2], Kevin P.M. Currie[3].
[1]Department of Anesthesiology, Vanderbilt University School of Medicine, Nashville, TN, USA, [2]Departments of Pharmacology and Psychiatry, Vanderbilt University School of Medicine, Nashville, TN, USA, [3]Departments of Anesthesiology and Pharmacology, Vanderbilt University School of Medicine, Nashville, TN, USA.
Adrenal chromaffin cells, an important neuroendocrine component of the sympathetic nervous system, maintain homeostatic and acute stress responses through secretion of catecholamines, neuropeptides, and other hormones. Mounting evidence links depression and cardiovascular diseases, and some